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Amylin Pharmaceuticals, Inc.

New claims:

1. An assay method for use in identifying or screening for compounds that stimulate or inhibit area postrema biological function, which comprises the steps of

(a) bringing together a test sample and an area postrema preparation, said test sample containing one or more test compounds;

(b) incubating said test sample and said area postrema preparation under conditions which would permit activation by said test compound of a biological process in, or the binding of said test compound to, said area postrema preparation; and

(c) identifying those test samples containing one or more test compounds which detectably activate, or bind to, said area postrema preparation.

2. The assay method of claim 1 which further comprises

(d) screening said test samples which detectably bind to said area postrema preparation for in vitro or in vivo stimulation or inhibition of area postrema mediated activity; and

(e) identifying those test samples which act as agonists or antagonists of said area postrema biological function.

3. The assay method of claim 1, wherein said area postrema preparation comprises isolated cells.

4. The assay method of claim 1, wherein said area postrema preparation comprises isolated membranes.

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5. The assay method of claim 1, wherein said area postrema preparation comprises isolated tissue.

6. The assay method of claim 1, wherein said test samples which detectably bind to said area postrema preparation are identified by measuring the displacement of a labeled first ligand from said area postrema preparation by said test sample, and comparing the measured displacement of said first labeled ligand from said area postrema preparation by said test sample with the measured displacement of said first labeled ligand from said area postrema preparation by one or more known second ligands.

7. The assay method of claim 1, wherein said test sample contains more than one test compound, which further comprises the steps of

(d) preparing two or more additional test samples from said test sample, said additional test samples being characterized in that they contain a lesser number of test compounds than said test sample from which they were prepared; and

(e) repeating steps (a)-(d) as many times as required until the test compound or compounds which activate, or bind to, said area postrema preparation have been identified.

8. The assay method of claim 2, wherein said test samples which detectably bind to said area postrema preparation are identified by measuring the displacement of a labeled first ligand from said area postrema preparation by said test sample, and comparing the measured displacement of said first labeled ligand from said area postrema preparation by said test sample with the measured displacement of said first

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labeled ligand from said area postrema preparation by one or more known second ligands.

9. The assay method of claim 8, wherein said test sample contains more than one test compound, which further comprises the steps of

(f) preparing two or more additional test samples from said test sample, said additional test samples being characterized in that they contain a lesser number of test compounds than said test sample from which they were prepared; and

(g) repeating steps (a)-(f) as many times as required until the test compound or compounds which bind to said area postrema preparation have been identified.

10. An assay method for determining the presence or amount of an area postrema binding compound in a test sample to be assayed for said compound, which comprises the steps of

(a) bringing together said test sample to be assayed and an area postrema preparation;

(b) measuring the ability of said test sample to compete against a labelled ligand for binding to said area postrema preparation; and, optionally,

(c) relating the amount of area postrema binding compound in said test sample with the amount of area postrema binding compound measured for a control sample in accordance with steps (a) and (b), said control sample being known to be free of any area postrema binding compound, and/or relating the amount of area postrema binding compound in said test sample with the amounts of area postrema binding compound measured for control samples containing known amounts of area postrema binding compound in accordance with steps (a) and (b), to

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determine the presence or amount of area postrema binding compound in said test sample.

11. A method for separating area postrema binding compounds from a sample, which comprises the steps of

(a) bringing together said sample and an area postrema preparation, said area postrema preparation comprising components of said area postrema bound to a solid carrier; and

(b) separating any area postrema binding compound which is bound to said area postrema preparation from the remainder of said test sample which is unbound.

12. A method for screening a biological substance for the presence of components of said area postrema, which comprises the steps of

(a) bringing together said biological substance with first area postrema binding compound;

(b) bringing together said biological substance with a second area postrema binding compound;

(c) optionally bringing together said biological substance with one or more additional area postrema binding compounds; and

(d) determining the relative binding affinities of said area postrema binding compounds for said area postrema preparation in said biological substance.

13. 14. A method of screening for a compound able to modulate a biological function of the area postrema related to fuel homeostasis, comprising adding a compound to an area postrema preparation, and measuring the effect on said biological function.

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14. The method of claim 13, wherein said area postrema preparation comprises one or more materials selected from the group consisting of area postrema, nucleus tractus solitarius material, and material from the dorsal motor nucleus of the vagus nerve.

15. The method of any of claims 13 or 14, wherein said material is selected from the group consisting of a membrane, a cell and a tissue.

16. The method of claim 13, wherein said biological function is modulation of pancreatic endocrine secretion.

17. The method of claim 13, wherein said biological function is modulation of body energy content.

18. The method of claim 13, wherein said biological function is linked to a metabolic disease.

19. The method of claim 18, wherein said metabolic disease is selected from the group consisting of diabetes and obesity.